

FIG. 1

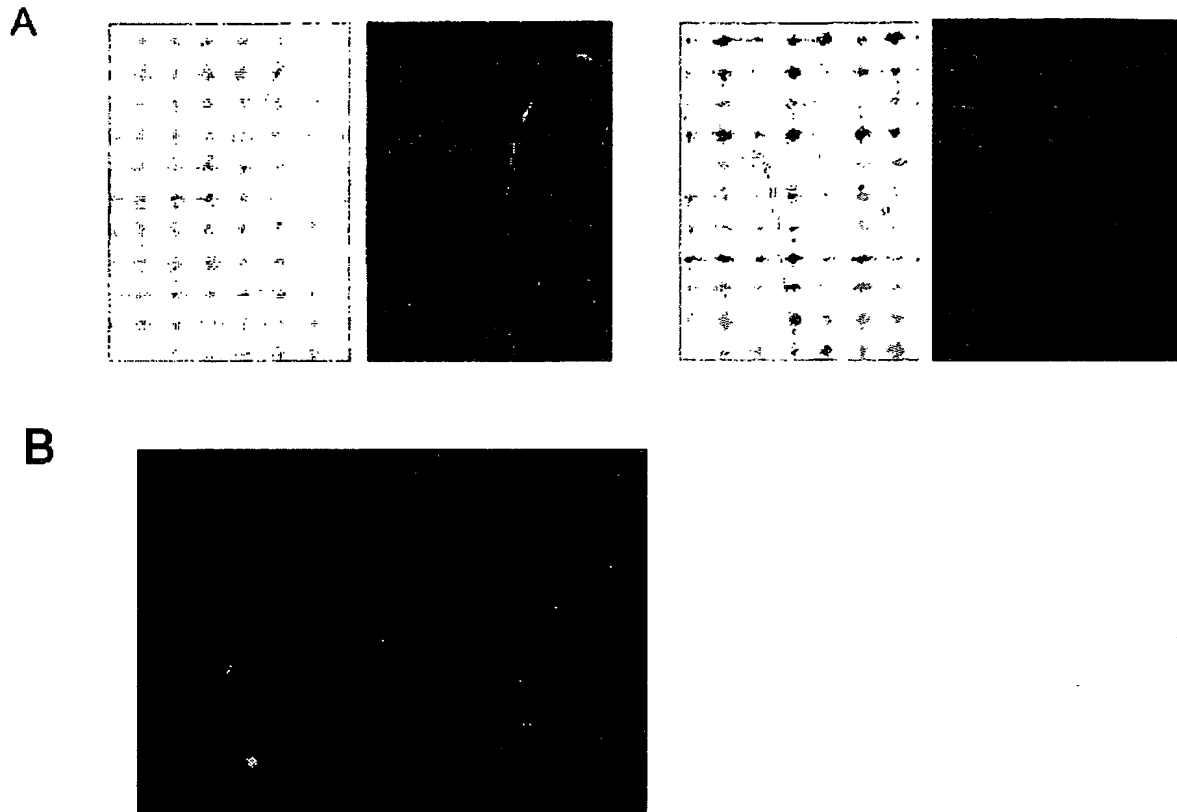


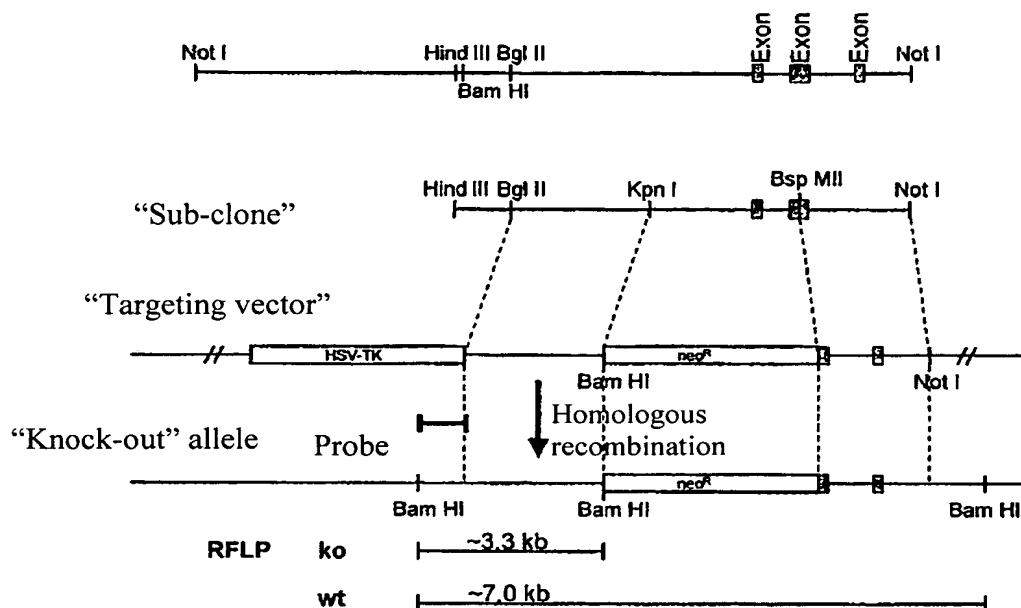
FIGURE 1: Expression of PMCA4 in isolated sperm cells

A: Mouse sperm cells reveal robust expression of PMCA4 in the acrosome and tail (shading, second image). A phase-contrast image of the isolated sperm is provided for comparative purposes. The two right-hand images show the negative control of the secondary antibody alone compared to the phase-contrast photograph of the two sperm cells.

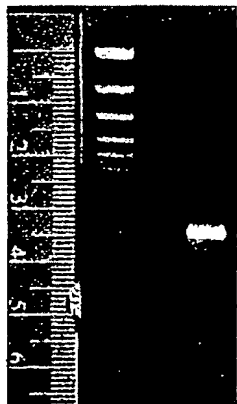
B: As with mouse sperm cells, human sperm cells also express PMCA4 in the acrosome and tail. The staining of the PMCA4 is illustrated here as an example.

FIG. 2

## A Phage insert (~12kb, wild-type allele)



## B M | ko | wt



## C

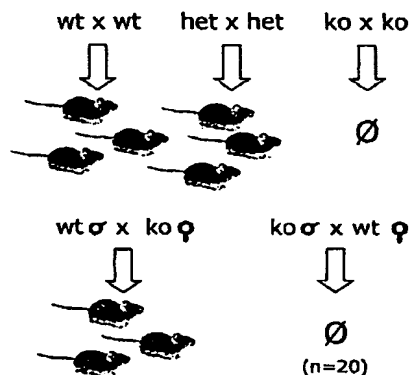


FIG. 2: Infertility in PMCA4-deficient mice.

A: A suitable strategy enabled the PMCA4 gene to be eliminated in the mouse. One complete exon and part of another exon were deleted by using homologous recombination, with the aid of a "targeting vector".

B: The gene manipulation resulted in a complete loss of gene expression in the PMCA4-deficient mice ("ko"), whereas normal mice ("wt") robustly express PMCA4 in sperm. M = Marker to determine the size of the RT-PCR product.

C: The male PMCA4-deficient animals (ko) obtained after genetic manipulation were found to be infertile, regardless of whether they were mated with normal females or with females that were also PMCA4-deficient.

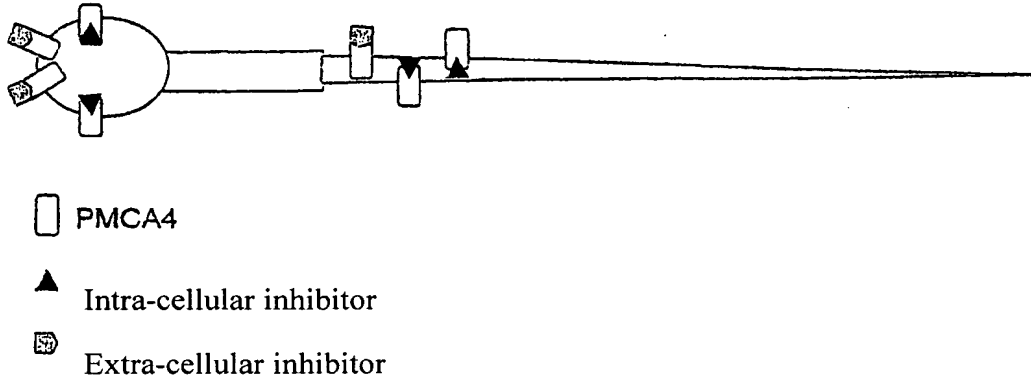


FIG. 3